VACCINE

FIELD OF THE INVENTION

The present invention relates to a carrier for introduction of substances into cells comprising a modified major capsid protein L1 of human papillomavirus (HPV-L1 protein) devoid of type-specific epitopes causing production of neutralising antibodies. The invention also includes an oligo- or polynucleotide coding for said carrier, vaccines comprising said carrier or said oligo- or polynucleotide, as well as methods of using the carrier or the oligo- or polynucleotide in vaccination against viral, bacterial or parasite infections as well as against development of certain cancers. Especially, infections of human papillomavirus and the development of cancer as a consequence of such infections are recognised.

BACKGROUND OF THE INVENTION

The Human Papillomavirus (HPV) is since long established as the major cause of cervical cancer (1), and has in recent years also been established as a cause of cancers of the penis, vulva, vagina, anus and orofarynx. There also exists indications that the virus may be involved in some cancers of the prostate, esophagus and in other head and neck cancers. HPV vaccine development is therefore a prime priority of preventive cancer research today (2).

The HPVs exist as >100 different types. Although types are defined by genetic homology, the genotypes have hitherto shown a strikingly good concordance with serotypes, i.e. hyperimmune antisera against one type will only neutralise the same type and not other genotypes. Cross-neutralisations have only been reported for certain closely related types and have had titers 2 orders of magnitude less than for the type-specific neutralisation (2,3).

The HPV capsid consists of 72 capsomers each containing 5 copies of the HPV major capsid protein L1. A minor capsid protein, L2, is present in much smaller amounts in the capsid (1:12 compared to the L1 protein) and the location of L2 is uncertain (2).

A number of small viruses express capsid proteins that when expressed self-assemble to form virus-like particles (VLPs) (i.e. particles morphologically similar to virus particles, but lacking the viral genome). The HPV major capsid protein L1 is among the best studied (2). HPV VLPs containing only L1 are morphologically similar to VLPs containing both L1 and L2 (2). Both particles with L1 only and particles with L1/L2 are highly efficient in eliciting a high-titered neutralising antibody response in several animal model systems (rabbits, cows, dogs and rhesus monkeys), even when injected in the absence of adjuvant (2).

Vaccination with papillomavirus VLPs has been shown to be highly efficient for protection, mediated by neutralising antibodies, against subsequent challenge with both cutaneous and mucosal papillomaviruses, but only in a type-specific manner (2). This strong type-specificity is surprising, since the major capsid protein of the HPVs is a highly evolutionarily conserved protein with very few amino acid changes between genetically related, but not cross-neutralising, HPV types.

The most common oncogenic HPVs are HPV16, 18, 31 and 45. HPV16 is found in about 50% of cervical cancers, HPV18 in about 20%, and these four types together correspond to >80% of all cervical cancers. Therefore, a commonly contemplated strategy is to manufacture vaccines containing HPV capsids of the 4 most common HPV types together (2).

Albeit this strategy appears likely to work for achieving significant cancer reduction, it has some distinct disadvantages. The formulation of vaccines containing 4 active components mixed together involves a

substantial additional cost in manufacturing and efficacy testing and quality control of each component.

Furthermore, some 10-20% of cervical cancers are caused by HPV types not included in the presently manufactured vaccine candidates. Apart from the fact that the vaccine could not possibly protect against these types, the possibility also exists that elimination of the 4 most common oncogenic HPV types may cause an increase in the prevalence of the other oncogenic HPV types, thus further diminishing the cancer-preventive gains. This latter scenario is, as predicted from population biology studies, likely to follow if there exists interference between different viral types. Several lines of indirect evidence do indicate that interference between HPV types does exist.

Several other HPV types cause significant morbidity and mortality, most notably HPV 6 and 11 that cause genital condylomas and recurrent respiratory papillomatoses, and HPVs 5 and 8 that cause cutaneous skin-cancers in the immunosuppressed host.In spite of the obvious advantages of broadly cross-reactive vaccines, the possibility to generate a broadly cross-reactive vaccine, by modifying the L1 protein to not contain immunodominant type-specific epitopes, has not been proposed. Several surface exposed and cross-reactive epitopes are exposed on papillomavirus particles (WO 96/33737), but are not immunogenic in the presence of the immunodominant type-specific epitope (4). Therefore, by modifying the L1 to remove immunodominant type-specific epitopes, it should be possible to generate a cross-reactive papillomavirus vaccine, using a modified HPV-L1 protein as a carrier of surface exposed HPV derived antibody epitopes.

Furthermore, VLPs are highly efficient in eliciting a cytotoxic T lymphocyte (CTL) response, and VLP vaccines have been reported to be highly efficacious (through a CD8+cell-dependent mechanism) in preventing and treating transplantable cancers in several mouse models, in spite

of the fact that immunization is made with an exogenous protein (5). The high immunogenicity appears to be due in part to the preservation of an active mechanism for infection of the cell (designated pseudo-infection, as no viral genome is introduced) which results in the capsid protein being processed and presented in the MHC class I presentation pathway (6). VLPs are therefore of general interest from a vaccine biotechnology point of view, since they can be used as a vehicle for efficient immunogenic delivery of any antigen (7).

Efficient immunisation using wild-type HPV VLPs carrying foreign antigens has been demonstrated in several systems, e.g. the MAGE melanoma antigens and human immunodeficiency virus antigens.

A potential problem using VPLs as vehicles for immunogenic delivery is blocking by type-specific neutralising antibodies. In Sweden 16% of the adult population are sero-positive for HPV-16, reflecting the importance of the problem. In addition, therapeutic vaccination is expected to require recurrent treatments, likely to induce a type-specific antibody response towards a wild-type VLP carrier.

Therefore, by modifying the L1 protein to remove type-specific epitopes causing production of neutralising antibodies, as has been described (8), and introduce antibody or T-cell epitopes in this carrier, it should be possible to generate an immunological response towards the introduced peptide, without obstruction from type-specific neutralising antibodies directed towards the carrier itself.

SUMMARY OF THE INVENTION

An object of the present invention is to provide means for preventing and treating viral, bacterial or parasite infections, especially of human papilloma virus, and the development of benign or malign consequences of such infections, as well as means for treating and preventing cancer.

The present invention provides for the use of a modified HPV-L1 protein devoid of type-specific epitopes causing production of neutralising antibodies, as a carrier of a substance into cells. As a result of the modification, this HPV-L1 protein carrier does not induce production of overt neutralising antibodies towards the carrier itself. In an embodiment of the invention, one or more amino acids may be deleted from said protein.

In particular, the invention provides for such an HPV-L1 protein in fusion with a peptide.

The invention also provides for such a carrier which is capable of giving rise to a protective antibody response, which antibody response may be cross-reactive towards two or more serologically defined subtypes of human papillomavirus.

The carrier must be physically coupled, that is fused, to the peptide for which it acts as a carrier, thus creating a fusion protein.

Particularly, peptides derived from HPV proteins and defining linear antibody epitopes and T-cell epitopes are recognised.

There is also envisaged combinations of said carrier with a minor coat protein of human papillomavirus (HPV-L2 protein), native or modified. Also this HPV-L2 protein can itself be fused to one or more further peptides.

The invention also provides for an oligo- or polynucleotide coding for said carrier. The invention makes it possible to create a better basis for eliciting an MHC class I mediated response, i.e. creating cytotoxic T-cells, without giving rise to type-specific neutralising antibodies towards the carrier, or without type-specific neutralising antibodies being present at the start.

It is also possible to use an HPV-L1 protein, modified as described above, as a carrier of oligo- or polynucleotides to cells.

DETAILED DESCRIPTION OF THE INVENTION

In one of its aspect, the invention provides for a carrier for introduction of a substance into cells, comprising a major capsid protein L1 of human papillomavirus (HPV-L1 protein) which has been intentionally modified to remove type-specific epitope(s) causing production of neutralising antibodies. In one preferred embodiment said HPV-L1 protein is in fusion with a peptide.

Preferably, said peptide comprises one or more T-cell epitopes, especially such epitopes derived from tumor, bacterial, parasite, viral or auto-antigens. In another preferred embodiment, said peptide comprises one or more antibody epitopes, such as tumor, bacterial, parasite, viral or auto-antigens, especially papillomavirus antigens.

The carrier can also be combined with a minor capsid protein L2 of human papillomavirus (HPV-L2 protein), which in its turn may be fused to one or more further peptides. These further peptides are e.g. T-cell or antibody epitopes, which may be derived from tumor, bacterial, parasite, viral or auto-antigens.

In a further embodiment the fusion protein is used as a carrier of oligo- or polynucleotides, e.g. such oligo- or polynucleotides which are coding for an antigen or an immunostimulatory (poly)peptide.

In another aspect, the invention provides for an oligo- or polynucleotide coding for the carrier as defined.

In further aspects, the invention provides for vaccines, comprising as an active ingredient a carrier or an oligo- or polynucleotide as defined above.

In further aspects of the invention there is provided methods of preventing or treating viral, bacterial or parasite infections by vaccination with a carrier or an oligo- or polynucleotide as defined above. In a preferred embodiment the infections is caused by papillo-

mavirus.

There is also provided methods of preventing or treating development of benign or malign consequences of human papillomavirus infection by vaccination with a fusion protein or an oligo- or polynucleotide as defined above.

In embodiments of the methods said human papillomavirus infection is warts or laryngeal papillomatosis.

Further aspects of the invention comprise methods of preventing or treating of cancer, including cancer of cervix, penis, vulva, vagina, anus and orofarynx, by vaccination with a fusion protein or an oligo- or polynucleotide as defined above.

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